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(54) Liver parenchymal cells having clonal growth ability, method for obtaining same, method for culturing same, and culturing system of primary hepatocytes

(57) The present invention provides liver parenchymal cells having a clonal growth ability, which possesses at least one of the cell biological properties such as: presence of peroxysome; being positive to hepatocyte-markers; being partially positive to neoplastic hepatocyte-markers or immature hepatocyte-markers; being positive to antibodies against the surface antigens of oval cells; and being partially positive to bile duct cell-markers. The present invention also provides a method

for obtaining such cells and a method for subculturing such cells.

With the liver parenchymal cells above, it will be possible to research in detail the process of development and differentiation, the mechanisms of growth and functional expression of hepatic cells, and to open up a new way to clarification of mechanisms of hepatoma and various other diseases and to development of therapeutic method against these diseases.



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EUROPEAN SEARCH REPORT

Application Number
EP 95 30 2398

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.)						
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim							
X	HAHM H. A. ET AL: "Primary culture of normal rat mammary epithelial cells within a basement membrane matrix. II. Functional differentiation under serum-free conditions" IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY, vol. 26, 1990, pages 803-814, XP002040994 *page 805, first column, second paragraph* ---	9-12	C12N5/06 C12N5/02						
X	MITAKA, T. ET AL: "Characteristics of small cell colonies developing in primary cultures of adult rat hepatocytes" VIRCHOWS ARCHIV B CELL PATHOLOGY INCLUDING MOLECULAR PATHOLOGY, vol. 62, no. 5, 1992, pages 329-335, XP002040995 * the whole document *	1							
X	BRILL S. ET AL: "Hepatic progenitor populations in embryonic, neonatal and adult liver" PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE, vol. 204, no. 3, 1993, pages 261-269, XP002040996 * the whole document *	1	TECHNICAL FIELDS SEARCHED (Int.Cl.) C12N						
X	NAKAGOMI, O. ET AL: "Morphological and functional studies of human fetal liver cells in primary monolayer culture" THE TOHOKU JOURNAL OF EXPERIMENTAL MEDICINE, vol. 132, 1980, pages 277-287, XP002040997 * the whole document *	1							
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<p>The present search report has been drawn up for all claims</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td style="width: 33%;">Place of search</td> <td style="width: 33%;">Date of completion of the search</td> <td style="width: 34%;">Examiner</td> </tr> <tr> <td>THE HAGUE</td> <td>17 September 1997</td> <td>Fernandez y Branas, F</td> </tr> </table> <p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document</p>				Place of search	Date of completion of the search	Examiner	THE HAGUE	17 September 1997	Fernandez y Branas, F
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Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.6)						
X	MOONEY, D. ET AL: "Switching from differentiation to growth in hepatocytes: Control by extracellular matrix" JOURNAL OF CELLULAR PHYSIOLOGY, vol. 151, 1992, pages 497-505, XP002040998 * the whole document *	1							
X,P	TATENO C. ET AL: "Clonal growth and differentiation of small size hepatocytes in the non-parenchymal cell fraction of rat liver" CELL STRUCTURE AND FUNCTION, vol. 19, no. 6, December 1994, page 552 XP002040999 * the whole document *	1-12							
X	WO 91 18087 A (Baylor College Medicine) 28 November 1991 * page 25 - page 26 *	9,10							
A	KREAMER B.L. ET AL: "Use of a low speed, iso-density percoll centrifugation method to increase the viability of isolated rat hepatocyte preparations" IN VITRO CELLULAR AND DEVELOPMENTAL BIOLOGY, vol. 22, no. 4, 1986, pages 201-211, XP002041008 * the whole document *	1-12	TECHNICAL FIELDS SEARCHED (Int.Cl.6)						
A,D	HIXSON, D.C. ET AL: "An antigenic portrait of the liver during carcinogenesis" PATHOBIOLOGY, vol. 58, no. 2, 1990, pages 65-77, XP002041001 * the whole document *	1-12							
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